

Effects of Yohimbine and Tolazoline on Isoproterenol and Angiotensin II-Induced Water Intake in Rats¹

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FREGLY, M. J., N. E. ROWLAND AND J. E. GREENLEAF. *Effects of yohimbine and tolazoline on isoproterenol and angiotensin II-induced water intake in rats.* BRAIN RES BULL 10(1)121-126, 1983.—Subcutaneous administration of the α_2 -adrenoreceptor antagonists, yohimbine and tolazoline, at doses up to 1000 $\mu\text{g}/\text{kg}$, had no effect on water intake of female rats. However, when these compounds were administered SC in combination with either the β -adrenoreceptor agonist, isoproterenol (10 to 25 $\mu\text{g}/\text{kg}$, SC), or with angiotensin II (200 $\mu\text{g}/\text{kg}$, SC), water intake was enhanced. In contrast, intraventricular administration of either tolazoline (10 and 20 $\mu\text{g}/\text{kg}$) or yohimbine (300 $\mu\text{g}/\text{kg}$) failed to augment the dipsogenic response to angiotensin II (150 $\mu\text{g}/\text{kg}$, SC). Thus, the enhancing effect of these α_2 -adrenoreceptor antagonists on isoproterenol- and angiotensin II-induced water intakes appears to be manifested peripherally, rather than centrally. In view of the fact that clonidine, an α_2 -adrenoreceptor agonist, has been shown to inhibit water intake induced by both isoproterenol and angiotensin II, the results suggest that the α_2 -adrenoreceptor may play a role in modulating water intake induced by these two dipsogenic agents.

| Rats | Thirst | Drinking | Yohimbine | Tolazoline | Angiotensin II | Isoproterenol |
|------|--------|----------|-----------|------------|----------------|---------------|
|------|--------|----------|-----------|------------|----------------|---------------|

ACUTE administration of clonidine, an α_2 -adrenergic agonist, inhibited the response to a number of dipsogenic stimuli including isoproterenol, 5-hydroxytryptophan, pilocarpine, hypertonic saline and dehydration [2, 3, 12]. The possibility exists that clonidine may exert its effect on water intake by virtue of its α_2 -adrenergic agonistic activity. The objective of the studies described here was to determine the effect of peripheral administration of two α_2 -adrenoreceptor blockers, yohimbine and tolazoline, on water intake of rats administered isoproterenol and angiotensin II subcutaneously. In addition, the effect of centrally administered tolazoline and yohimbine on the dipsogenic effect of peripherally administered angiotensin II was tested. If α_2 -adrenoreceptors play a role in the response to dipsogenic stimuli, then blockade of these receptors should result in effects on drinking opposite to those observed when the α_2 -adrenoreceptors are stimulated by clonidine. The results suggest that peripherally, but not centrally, administered blockers amplified the dipsogenic effects of angiotensin II.

GENERAL METHOD

Naive female rats of the Blue Spruce Farms (Sprague Dawley) strain were used. They were kept three per cage in a room maintained at $26 \pm 1^\circ\text{C}$ and illuminated from 6 a.m. to 6 p.m. All rats received Purina Laboratory Chow and tap water ad lib prior to the studies.

At the beginning of each study (9 a.m.) the rats were divided randomly into the appropriate groups, and weighed. The compounds to be tested were then administered. Each rat was placed in a cage by itself without food and given a preweighed bottle of distilled water (26°C). Water intake was then measured hourly for the next two hours by weighing each bottle to the nearest 0.1 g. The fluid containers consisted of infant nursing bottles with cast bronze fountains [5].

EXPERIMENT 1: EFFECT OF YOHIMBINE ALONE, AND IN COMBINATION WITH ISOPROTERENOL, ON WATER INTAKE OF RATS

Study 1

Twenty-four rats (300 to 350 g) were divided randomly into four equal groups and weighed. Groups 1 to 3 received yohimbine (Sigma Chemical Co.) SC at 250, 500 and 1000 $\mu\text{g}/\text{kg}$ respectively. Group 4 served as control group and was injected SC with the distilled water vehicle.

Study 2

On the day of the study, 24 rats (250 to 300 g) were divided into four equal groups and weighed. Groups 1, 2, and 3 were administered 150, 300 and 600 μg yohimbine/kg SC, respectively, in combination with 10 μg of isoproterenol/kg SC. Group 4 received 10 μg of isoproterenol/kg SC in combination with the vehicle used to dissolve yohimbine.

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Study 3

After a lapse of one week, the rats from Study 2 were used a second time. Study 3 was carried out identically to Study 2 except that 25 µg of isoproterenol/kg was administered SC and the doses of yohimbine used were 250, 500 and 1000 µg/kg, SC. Treatments were randomized to assure that the rats in this study did not receive the same treatment as in Study 2.

Statistical analysis of data was carried out by means of a one-way analysis of variance (Study 1) and a two-way analysis of variance (Studies 2 and 3) [10].

EXPERIMENT 2: EFFECT OF TOLAZOLINE ALONE, AND IN COMBINATION WITH ISOPROTERENOL, ON WATER INTAKE OF RATS

Study 1

Twenty-four naive rats (250 to 300 g) were divided randomly into four equal groups. Groups 1 to 3 received tolazoline SC at 250, 500 and 1000 µg/kg, respectively. Group 4 served as a control group and was injected with the vehicle used to dissolve the tolazoline.

Study 2

Twenty-four naive rats (250–300 g) were divided randomly into 4 equal groups. Groups 1, 2 and 3 received 250, 500 and 1000 µg of tolazoline/kg SC in combination with 15 µg of isoproterenol/kg SC. Group 4 was administered 15 µg of isoproterenol/kg SC, in combination with the vehicle used to dissolve the tolazoline.

Study 3

After a lapse of one week, the rats used in Study 2 were also used here. Study 3 was carried out identically to Study 2 excepting that 25 µg of isoproterenol/kg was administered SC.

Statistical analysis of these studies was carried out as described in Experiment 1.

EXPERIMENT 3: EFFECT OF YOHIMBINE ON ANGIOTENSIN II-INDUCED WATER INTAKE IN RATS

Twenty-one naive female rats (220 to 250 g) were divided into 3 equal groups. All 3 groups received angiotensin II (200 µg/kg, SC). Groups 1 and 2 received in addition 150 and 300 µg yohimbine/kg SC while Group 3 received an equal volume of distilled water used to dissolve the yohimbine.

Statistical analysis of the results was carried out by a one-way analysis of variance [10].

EXPERIMENT 4: EFFECT OF CENTRALLY ADMINISTERED TOLAZOLINE AND YOHIMBINE ON ANGIOTENSIN II-INDUCED DRINKING

Study 1

Sixteen naive rats (225 to 275 g) were divided into two equal groups. Each rat was surgically prepared with an indwelling intracerebroventricular cannula. Using Equithesin (1.5 ml/kg) for anesthesia and a Kopf stereotaxic instrument, a 23 gauge stainless steel tube (11 mm long) with a wire obturator was implanted to end at the dorsal part of the right lateral cerebral ventricle. The cannula was secured with skull screws and dental cement, and a post-operative recovery time of 1 week was allowed prior to testing.

TABLE 1
EFFECT OF YOHIMBINE (SC) ON WATER INTAKE OF FEMALE RATS (6 PER GROUP) DURING TWO HOURS AFTER TREATMENT

| Experimental Group | Mean Body wt. (g) | Cumulative Water Intake (ml/kg body wt) during: | |
|---------------------------|-------------------|---|-----------|
| | | 1 hr | 2 hr |
| Control | 320 ± 5* | 3.9 ± 0.9 | 4.2 ± 0.9 |
| Yohimbine (250 µg/kg) | 306 ± 4 | 4.0 ± 1.2 | 4.9 ± 1.2 |
| Yohimbine (500 µg/kg) | 321 ± 12 | 2.9 ± 0.7 | 6.8 ± 1.6 |
| Yohimbine (1000 µg/kg) | 322 ± 6 | 2.0 ± 0.8 | 3.6 ± 1.0 |

*One standard error of mean.

Intracranial injections of either tolazoline (10 µg/kg, 8 rats) or isotonic saline (8 rats) were performed with the animals held gently in a towel. The wire obturator was removed, and an injector cannula (11.5 cm long, 27 gauge) inserted to protrude just beyond the end of the implanted cannula, i.e., into the lumen of the ventricle. The inner tube was connected, via PE10 tubing, to a 25 µl syringe. A 5 µl volume was injected manually over a period of 5 to 10 sec. The injector was removed 5 sec later, the obturator replaced, and the animal placed immediately into an individual test cage. Fifteen minutes later all rats were administered angiotensin II at a dose of 150 µg/kg SC. A preweighed bottle of water was placed on each cage and water intakes were measured at one-half and one hour after administration of angiotensin II.

Study 2

This study was identical to Study 1 except that 20 µg of tolazoline/kg was administered IVT and that there were four rats per group.

Study 3

This study was also identical to Study 1 except that yohimbine (300 µg/kg) was administered IVT and that there were 5 rats per group.

RESULTS*Experiment*

Yohimbine administered SC to rats at 250, 500 and 1000 µg/kg had no significant effect on water intake during either the first or second hours after administration (Table 1).

When 300 µg yohimbine/kg was administered in combination with 10 µg of isoproterenol/kg, water intake during the first hour increased significantly ($p < 0.05$) above that of the group receiving only isoproterenol (Fig. 1). Although all three groups treated with yohimbine had greater water intakes during the first and second hours than the group treated with isoproterenol alone, the differences in water intakes from control were not statistically significant save only for the 300 µg dose of yohimbine during the first hour.

When yohimbine was administered in combination with a

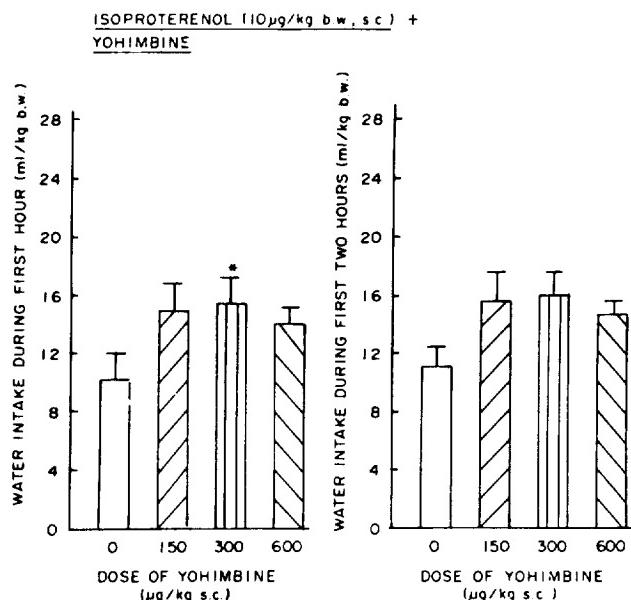


FIG. 1. The effect of graded doses of yohimbine on water intake in response to 10 μg isoproterenol/kg is shown during the first and first two hours after treatment. One standard error is set off at each mean. * $p<0.05$ compared with control (0 dose) group.

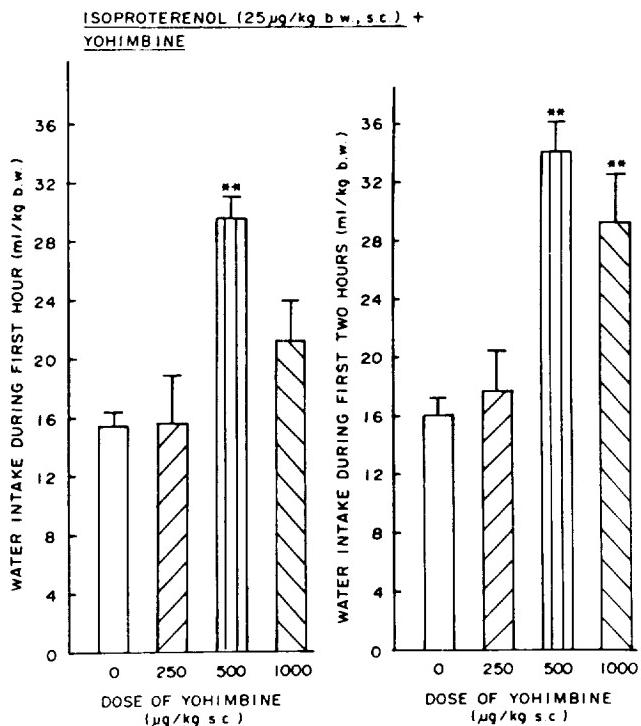


FIG. 2. The effect of graded doses of yohimbine on water intake in response to 25 μg isoproterenol/kg is shown during the first and first two hours after treatment. One standard error is set off at each mean. ** $p<0.01$ compared with control (0 dose) group.

TABLE 2
EFFECT OF TOLAZOLINE (SC) ON WATER INTAKE OF FEMALE RATS (6 PER GROUP) DURING 2 HOURS AFTER TREATMENT

| Experimental Group | Mean Body wt. (g) | Cumulative Water Intake (ml/kg body wt) during: | |
|--|-------------------|---|---------------|
| | | 1 hr | 2 hr |
| Control | 233 \pm 4* | 1.2 \pm 0.3 | 2.4 \pm 0.7 |
| Tolazoline (250 $\mu\text{g}/\text{kg}$) | 228 \pm 5 | 1.0 \pm 0.2 | 1.6 \pm 0.2 |
| Tolazoline (500 $\mu\text{g}/\text{kg}$) | 234 \pm 6 | 1.1 \pm 0.1 | 1.4 \pm 1.1 |
| Tolazoline (1000 $\mu\text{g}/\text{kg}$) | 242 \pm 8 | 1.5 \pm 0.4 | 1.8 \pm 0.4 |

*One standard error of mean.

larger dose of isoproterenol (25 $\mu\text{g}/\text{kg}$), water intake of the group receiving 500 $\mu\text{g}/\text{kg}$ was significantly greater than that of the control group. During the second hour, the water intakes of the groups receiving the two higher doses of yohimbine were significantly greater ($p<0.01$) than that of controls receiving only isoproterenol (Fig. 2).

Experiment 2

Tolazoline administered SC to rats at 250, 500 and 1000 $\mu\text{g}/\text{kg}$. SC had no significant effect on water intake during either the first or second hours after administration (Table 2).

When tolazoline was administered in combination with 15 μg isoproterenol/kg, water intakes during the first and second hours after treatment were significantly greater than that of the group receiving only isoproterenol (Fig. 3). The group receiving 250 μg tolazoline/kg had a water intake not significantly different from the control group but significantly different ($p<0.01$) from the group treated with 500 μg of tolazoline/kg.

When tolazoline was administered in combination with 25 μg isoproterenol/kg water intake of the control group was only slightly greater than it was following administration of 15 μg isoproterenol/kg (Fig. 4). Administration of tolazoline increased water intake above that of the group receiving only isoproterenol during both hours after treatment, but the intakes were significantly greater only during the second hour and only for the group receiving 500 μg of tolazoline/kg.

Experiment 3

Administration of yohimbine increased the angiotensin II-induced drinking response of rats (Fig. 5). Administration of 300 μg of yohimbine/kg was accompanied by an increase in water intake that was significantly ($p<0.05$) greater than that of the group administered angiotensin II alone. Water intake during the first two hours after administration of angiotensin II was only slightly greater than that observed during the first hour. Thus, the major portion (85–95%) of the two hour water intake occurred during the first hour of the experiment. This was also the case in Experiments 1 and 2.

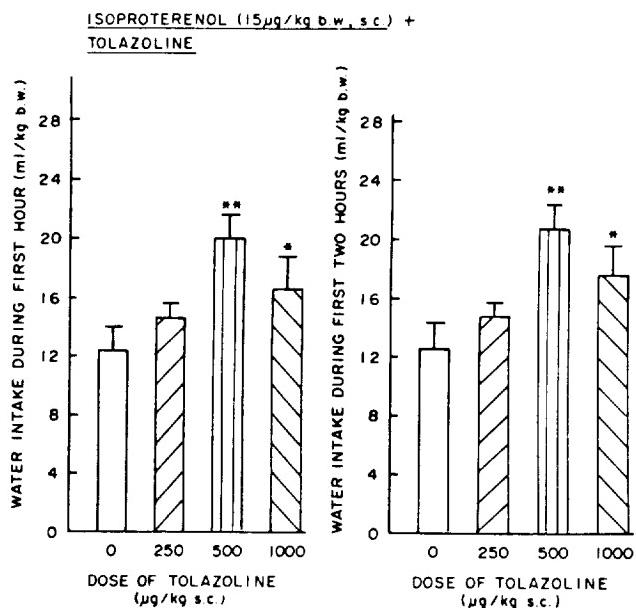


FIG. 3. Effect of graded doses of tolazoline on water intake in response to $15 \mu\text{g}$ isoproterenol/kg is shown during the first and first two hours after treatment. One standard error is set off at each mean. * $p < 0.05$; ** $p < 0.01$ compared with control (0 dose) group.

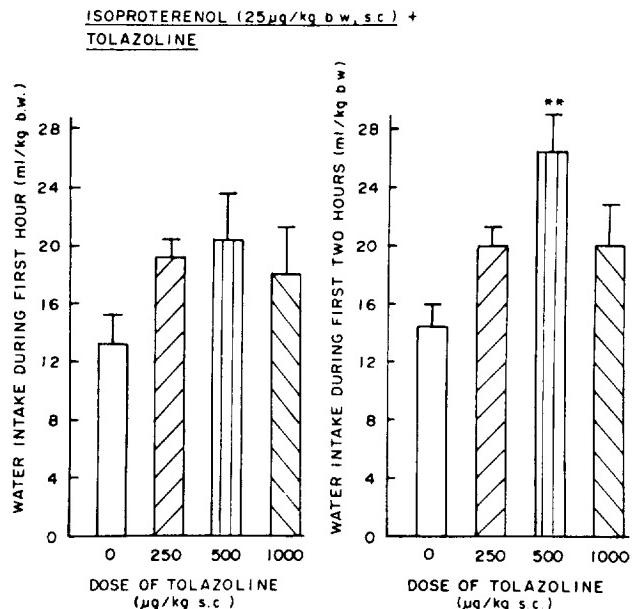


FIG. 4. Effect of graded doses of tolazoline on water intake in response to $25 \mu\text{g}$ isoproterenol/kg is shown during the first and first two hours after treatment. One standard error is set off at each mean. ** $p < 0.01$ compared with control (0 dose) group.

Experiment 4

Peripheral administration of angiotensin II was accompanied by a vigorous water intake that occurred within one-half hour after treatment (Table 3). Intraventricular (IVT)

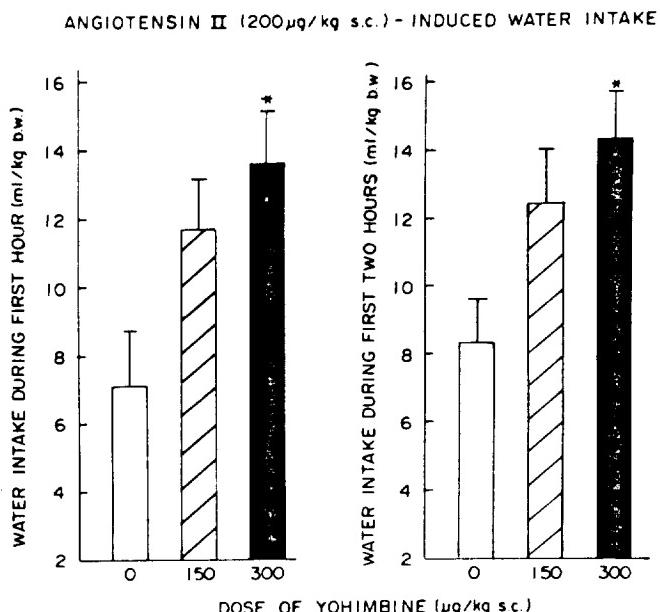


FIG. 5. Effect of graded doses of yohimbine on water intake in response to $200 \mu\text{g}$ angiotensin II/kg is shown during the first and first two hours after treatment. One standard error is set off at each mean. * $p < 0.01$ compared with control (0 dose) group.

administration of tolazoline at either 10 or $20 \mu\text{g}/\text{kg}$ 15 minutes prior to angiotensin II had no significant effect on the angiotensin II-induced drinking response. An additional experiment in which yohimbine ($300 \mu\text{g}/\text{kg}$) was administered IVT 15 minutes prior to angiotensin II also failed to affect the drinking response to peripherally administered angiotensin II.

DISCUSSION

Clonidine, an α_2 -adrenergic agonist, has been shown to inhibit dipsogenic responses to administration of isoproterenol, 5-hydroxytryptophan, angiotensin II and Pilocarpine, as well as to administration of hypertonic saline and a 24 hour period of dehydration [2, 3, 12]. Activation of α_2 -adrenoreceptors by clonidine is reported to lead to depression of norepinephrine release by sympathetic nerves [11]. The mechanisms by which this may have inhibited the dipsogenic responses to angiotensin II, hypertonic saline and dehydration, while not clearly understood, may be centrally located. On the other hand, the inhibition of the drinking response to isoproterenol, 5-hydroxytryptophan and pilocarpine may be explained both by the ability of clonidine to suppress cAMP formation peripherally and by its inhibitory effect on the release of renin from the kidney [9]. Angiotensin II, formed as a result of administration of these compounds, is believed to be the ultimate dipsogenic agent for them [1].

Yohimbine and tolazoline are reported to be selective presynaptic α_2 -adrenoreceptor antagonists which are believed to facilitate the release of norepinephrine by sympathetic nerves [11]. It was therefore of interest to determine their effect on the dipsogenic response to acute administration of isoproterenol and angiotensin II. Neither yohimbine

TABLE 3
EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF TOLAZOLINE AND YOHIMBINE ON WATER INTAKE OF RATS INDUCED BY PERIPHERALLY ADMINISTERED ANGIOTENSIN II

| Experimental Group | No. of Rats | Mean Body wt. (g) | Cumulative Water Intake (ml/kg body wt) during: | |
|--|-------------|-------------------|---|----------------|
| | | | 1 hr | 2 hr |
| Study 1 | | | | |
| Angiotensin II (150 μ g/kg, SC) | 8 | 236 \pm 11* | 19.6 \pm 2.5 | 20.1 \pm 2.4 |
| Angiotensin II + Tolazoline (10 μ g/kg, IVT) | 8 | 269 \pm 10 | 15.8 \pm 2.5 | 19.2 \pm 3.3 |
| Study 2 | | | | |
| Angiotensin II (150 μ g/kg, SC) | 4 | 287 \pm 7 | 22.4 \pm 3.8 | 23.1 \pm 3.8 |
| Angiotensin II + Tolazoline (20 μ g/kg, IVT) | 4 | 267 \pm 8 | 16.1 \pm 1.8 | 18.8 \pm 3.6 |
| Study 3 | | | | |
| Angiotensin II (150 μ g/kg, SC) | 5 | 289 \pm 10 | 13.6 \pm 1.0 | 14.8 \pm 0.8 |
| Angiotensin II + Yohimbine (300 μ g/kg, IVT) | 5 | 304 \pm 11 | 13.0 \pm 2.1 | 15.1 \pm 2.0 |

*One standard error of mean.

nor tolazoline, administered alone in graded doses, had an effect on water intake during two hours after treatment (Tables 1 and 2). Similar results have been reported by others [4,6]. However, when administered with either isoproterenol or angiotensin II, the dipsogenic response was augmented (Figs. 1-5). These results suggest that release of norepinephrine may be important in modulating the response to dipsogenic stimuli. Those substances that act to facilitate the release of norepinephrine from sympathetic nerve endings, such as α -adrenolytic compounds, may also facilitate the drinking response to administered isoproterenol [11]. Such an effect has been reported for phentolamine [7,8]. On the other hand, those substances that act to attenuate the release of norepinephrine from sympathetic nerve endings, such as clonidine, inhibit the drinking response to isoproterenol. A possibility exists that the α_2 -adrenoreceptor is involved with thirst and drinking on a broader scale since all dipsogenic stimuli tested thus far, whether of extracellular or cellular origin, can be inhibited by clonidine [3].

α_2 -Adrenoreceptors which bind clonidine, yohimbine and tolazoline with a relative high degree of selectivity are located not only presynaptically but also postsynaptically in the central nervous system and in some peripheral tissues. Hence, it was important to determine whether centrally

(IVT) administered tolazoline and yohimbine could augment the dipsogenic response to peripherally administered angiotensin II. At the doses used, tolazoline and yohimbine given IVT failed to augment the drinking response to angiotensin II. We believe the doses of both compounds were adequate. However, the possibility exists that the compounds might not have had as ready access to their site of action when administered IVT as when administered peripherally.

Alternatively, the results suggest that augmentation of the release of norepinephrine from nerve endings peripherally may be responsible for the enhanced drinking response to treatment with tolazoline and yohimbine. Thus, augmented release of norepinephrine could induce release of renin from the kidneys and result in the formation of angiotensin II and enhancement of the dipsogenic effect of exogenously administered angiotensin II. Evidence for this possibility must await additional studies.

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